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EFFECTS OF ACUTE EXPOSURE TO KARATE (5% EC LAMBDA-CYHALOTHRIN) ON HAEMATOLOGICAL AND HISTOPATHOLOGICAL PARAMETERS OF *PANGASIANODON HYPOPHTHALMUS*

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ABSTRACT

Aquatic contamination caused by pesticide runoff has become a major ecological concern due to its harmful effect on fish health. The present study evaluated the effects of acute lambda-cyhalothrin exposure on the haematological and histopathological parameters of *Pangasianodon hypophthalmus*. Based on a previously determined 96-h LC value of 0.08 ppm, fish were exposed to 0.04 ppm and 0.08 ppm lambda-cyhalothrin for 96 h.Haematological parameters, including total erythrocyte count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and total leucocyte count were analyzed. Histopathological alterations in gill, liver and kidney tissues were also analyzed. The results revealed significant reduction in haemoglobin, packed cell volume and total leucocyte count in exposed groups compared to control, along with alterations in erythrocyte indices, which indicate a disturbance in normal haematological function under toxic stress. Histopathological observation revealed alterations such as lamellar fusion and epithelial lifting in gills, vacuolar degeneration in liver and tubular degeneration in kidney tissues. The severity of tissue damage was concentration dependent. The findings suggest that acute exposure to lambda-cyhalothrin induces significant haematological and histopathological alterations in *Pangasianodon hypophthalmus*, implyingits potential risk to aquatic organisms.

KEYWORDS: Lambda-cyhalothrin, Pangasianodon hypophthalmus, acute toxicity, haematology, Histopathology

INTRODUCTION

Pesticides are substances of natural or synthetic origin that are used globally to control, regulate, reduce or mitigate harmful organisms in agricultural processes (WHO, 2010). With the expansion of modern agricultural practices, large quantities of chemicals, including pesticides, fungicides, and insecticides, are released into the environment (Stoytcheva, 2011). These pesticide residues enter aquatic systems by leaching, runoff, precipitation, percolation, aerial drift or accidental spills. In addition to contamination, these pesticides also alter the physiochemical integrity of aquatic systems and thus cause adverse effects by disrupting the food chain and altering the habitat (Olutona *et al*., 2016). Some of these pesticides may be non-degradable and thus accumulate in living systems and cause harm to non-target species by moving up the food chain (Saha and Kaviraj, 2008). Humans largely depend on aquatic ecosystems and therefore come in contact with these chemicals either directly or indirectly (Singh *et al*., 2015). Several studies have reported the adverse effects of acute and chronic pesticide exposure on aquatic organisms (Bartosz *et al*., 2018; Ullah *et al*., 2019). The toxicity of pesticides depends on the pesticide formulation, species sensitivity, and water quality parameters.

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Pyrethroids are synthetic insecticides commonly used in modern agricultural practices due to their effective insecticidal properties and relatively lower toxicity to mammals, although they are highly toxic to aquatic organisms, especially fish (Prusty *et al*., 2015). Lambda-cyhalothrin is a type II pyrethroid, which is known for its broad-spectrum application in agricultural and household pest management as well as in public and animal health for disease control (Dey and Saha, 2014). It is lipophilic and has a strong affinity for adsorption onto sediments and organic matter. It acts as a potent neurotoxin by interfering with the normal functioning of sodium channels in the nervous system, leading to membrane depolarization and neuronal hyperexcitability (Ullah *et al*., 2019). Due to its lipophilic nature, rapid absorption, high bioaccumulation potential, andability to interfere with neuronal sodium channels, lambda-cyhalothrin is considered highly toxic to fish (Xu *et al.,* 2023). Due to its extensive agricultural application and potential entry into aquatic systems, lambda-cyhalothrin has become a major concern in aquatic toxicology studies (Yao *et al*., 2024).

Pesticide contamination in aquatic ecosystems can cause harmful effects on physiology, growth and survival of fish and other aquatic organisms (Abdel-Latif *et al*., 2023). Haematological and biochemical alterations are commonly recognized as sensitive indicators of toxic stress in fish exposed to environmental contaminants (Vali *et al*., 2022). Parameters such as total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV) and total leukocyte count (TLC) are widely used to assess the physiological condition of fish under stress (Singh and Srivastava, 2010). Likewise, histopathological examination of vital organs serves as an effective tool for evaluating tissue-level damage caused by toxic substances (Abdel-Latif *et al*., 2023). Since gills remain in direct contact with surrounding water, they are particularly vulnerable to aquatic pollutants (Au, 2004), while structural alterations in kidney and liver tissues are also frequently associated with pesticide exposure (Barhoumi and Messaoudi, 2012). Therefore, haematological and histopathological analyses provide important information regarding the toxic effects of pesticides in aquatic ecosystems.

The striped catfish (*Pangasianodon hypophthalmus*), a member of the family Pangasiidae, was selected as the experimental species for the present study. It is one of the most commonly cultured freshwater fish in many Asian countries (Abd-Elaziz *et al*., 2023). It contributes approximately 3% of total global aquaculture production in 2016 (FAO, 2018). Due to its high growth rate, ability to tolerate fluctuating environmental conditions, palatable flavour, affordable price, high market demand and substantial profitability it is a highly preferred species for aquaculture (De Silva and Phuong, 2011). However, limited information is available regarding the acute effects of lambda-cyhalothrin on the haematological and histopathological characteristics of this species.

Therefore, the present study aimed to evaluate the effects of acute lambda-cyhalothrin exposure on the haematological and histopathological parameters of *Pangasianodon hypophthalmus*.

MATERIALS AND METHODS

Healthy *Pangasianodon hypophthalmus* (15 ± 3 cm in length and 50 ± 5 g in body weight) were procured from a local fish vendor in Thiruvananthapuram district, Kerala, India.The fish were transported to the laboratoryand acclimatized for 2 weeks, during which they were fed twice daily with commercial pellet food (35% protein). The water quality parameters were determined using the standard method by APHA (2023). The pesticide used for the study was Karate (Lambda-cyhalothrin 5% EC), purchased from Syngenta India Ltd, Pune, Maharashtra, India.

Acute toxicity studywas conducted in accordance with OECD Test Guideline 203 (OECD, 2019). Glass tanks (72 L capacity; 60 × 40 × 30 cm) containing 50 L of dechlorinated water were used. Prior to exposure, the fish were starved for 24hrs and were not fed during the experimental period. Based on the previously determined 96-h LC_{50} value of 0.08ppm for lambda-cyhalothrin, two exposure concentrations, 0.04ppm (½ of LC_{50}) and 0.08 ppm (LC_{50}), were selected. The experimental setup consisted of one control tank and two experimental tanks, each maintained in triplicate with 10 fish of uniform size and weight per tank. At the end of the 96 h exposureperiod, blood and tissue samples were collected for further analyses.

Haematological parameters including total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total leucocyte count (TLC) were

analyzed. Blood samples were collected from the caudal vein and transferred to EDTA-coated tubes for haematological analysis. Total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin concentration (Hb), and packed cell volume (PCV) were determined following standard haematological procedures described by Dacie and Lewis (2012). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae described by Noga (2010).

For histopathological analysis, gill, liver and kidney tissues from the control and exposed groups were dissected and fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated through a graded series of ethanol, cleared in xyleneand embedded in paraffin wax. Sections of 5 µm thickness were prepared using a rotary microtome and stained with haematoxylin and eosin for microscopic examination (Bancroft and Gamble, 2008).

Data obtained for TEC, Hb, PCV, MCV, MCH, MCHC, and TLC were subjected to one-way analysis of variance (ANOVA). Differences among treatment groups were evaluated by Duncan's Multiple Range Test (DMRT) as a post hoc analysis. Statistical significance was considered at $p < 0.05$. All data are presented as mean ± standard deviation (SD).

The experimental protocol involving fish was approved by the Institutional Animal Ethics Committee (IAEC), Department of Biochemistry, University of Kerala, Thiruvananthapuram, India (Proposal No. IAEC-1-KU-6/2025-ZOO-SRP (16)).

RESULTS

The physicochemical properties of water, including temperature, pH, dissolved oxygen, salinity and hardness, were monitored throughout the study. The mean ± SD values recorded were 24.53 ± 0.49 °C, 6.60 ± 0.35, 7.33 ± 0.06 mg L^{-1}, 0.05 ± 0.00 ppt and 176.67 ± 5.77mg L^{-1} respectively.

A lethal concentration (0.08 ppm) and a sublethal concentration (0.04 ppm) of lambda-cyhalothrin were selected for the haematological and histopathological assessment. The effects of 96-h exposure to lambda-cyhalothrin on haematological parameters, including total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and total leucocyte count (TLC), are presented in Table 1. One-way analysis of variance revealed significant differences ($P < 0.05$) among treatment groups for TEC, Hb, PCV, MCV, MCH, and TLC, whereas MCHC did not differ significantly ($P > 0.05$).

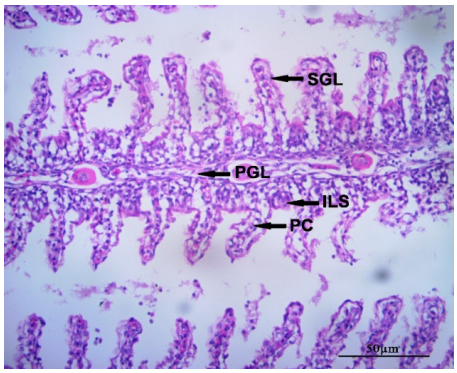

Fig. 1. Histologicalsections of the gill of *Pangasianodon hypophthalmus* (control)

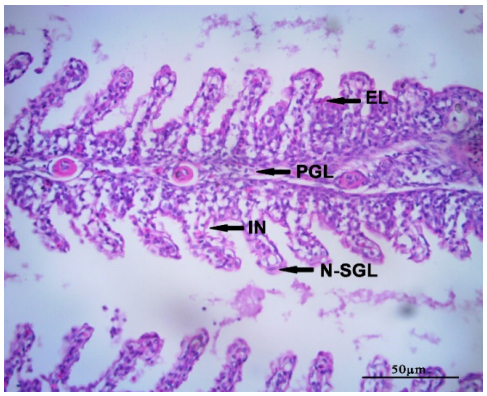

Fig. 2. Histologicalsection of the gills of *Pangasianodon hypophthalmus* exposed to 0.04 ppm lambda-cyhalothrin.

Fig. 3. Histological section of the gills of *Pangasianodon hypophthalmus* exposed to 0.08 ppm lambda-cyhalothrin.

TEC showed an overall reduction in exposed fish, with the lowest value recorded at 0.08 ppm. Haemoglobin concentration decreased significantly with increasing lambda-cyhalothrin concentration, indicating a concentration-dependent response. Similarly, PCV values were significantly lower in both treated groups than in the control. MCV exhibited significant alterations following pesticide exposure; however, the changes did not follow a concentration-dependent pattern. Likewise, MCH differed significantly among the treatment groups but showed no consistent concentration-dependent trend. Although MCHC values exhibited a slight increase in exposed fish compared with the control, the differences were not statistically significant ($P > 0.05$). TLC was significantly reduced in both treatment groups compared with the control, although the values did not decrease progressively with increasing concentration.

Histological sections of the gills from the control fish showed normal primary gill lamellae (PGL) and secondary gill lamellae (SGL) (Fig. 1). The secondary gill lamellae showed central pillar cells (PC) lined by the respiratory epithelium, and the interlamellar spaces (ILS) were clearly visible. In the gill sections of fish exposed to 0.04 ppm lambda-cyhalothrin, the secondary gill lamellae showed necrosis (N-SGL) and inflammation (IN). The interlamellar spaces exhibited epithelial lifting (EL) (Fig. 2). Gills of fish exposed to 0.08 ppm lambda-cyhalothrin showed oedematous and club-shaped secondary gill lamellae (E-SGL) with epithelial lifting (EL) (Fig. 3).

Sectionsof the liver from the control fish showed normal hepatocytes (NH) and hepatopancreas (HP) (Fig. 4), whereas liver sections from fish exposed to 0.04 ppm lambda-cyhalothrin showed disarrangement of hepatic cords, cytoplasmic vacuolation (VH), and nuclear pyknosis (NP) (Fig. 5). Disarrangement of hepatic cords was also observed in the liver sections of fish exposed to 0.08 ppm lambda-cyhalothrin. In addition, the cytoplasm of hepatocytes showed vacuolation (VH), and the sinusoidal spaces exhibited mild haemorrhage (MH) (Fig. 6).

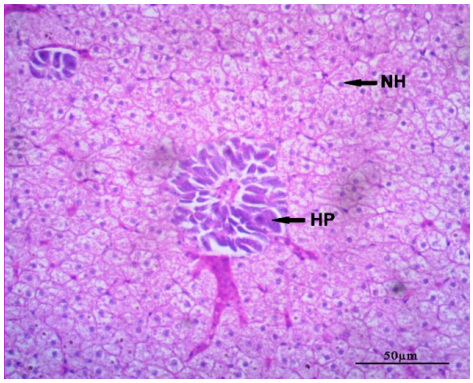

Fig. 4. Histological section of the liver of *Pangasianodon hypophthalmus* (control).

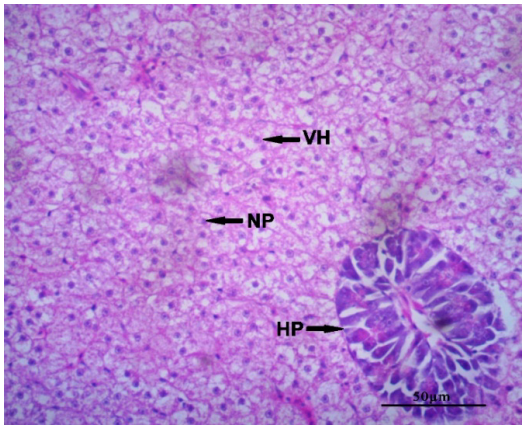

Fig. 5. Histological section of the liver of *Pangasianodon hypophthalmus* exposed to 0.04 ppm lambda-cyhalothrin.

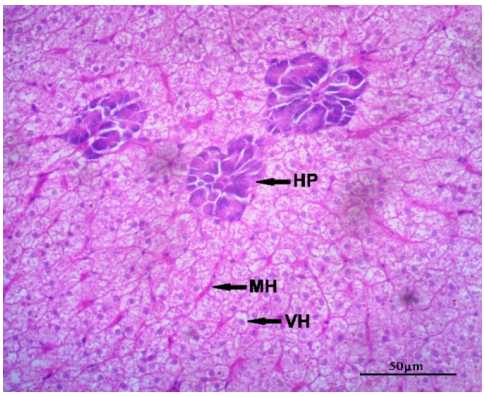

Fig. 6. Histological section of the liver of *Pangasianodon hypophthalmus* exposed to 0.08 ppm lambda-cyhalothrin.

Control fish kidney sections showed normal renal corpuscles consisting of Bowman's capsule (BC) enclosing the glomerulus. The renal tubules exhibited normal histology (Fig. 7). Renal tubules showed oedema and moderate degeneration (DRT) in sections of fish exposed to 0.04 ppm lambda-cyhalothrin. The renal interstitial showed moderate haemorrhage (HE) (Fig. 8). In fish exposed to 0.08 ppm lambda-cyhalothrin, the renal tubules and Bowman's capsule were swollen and oedematous (DRT, EB). The interstitial also showed moderate haemorrhage (HE) (Fig. 9).

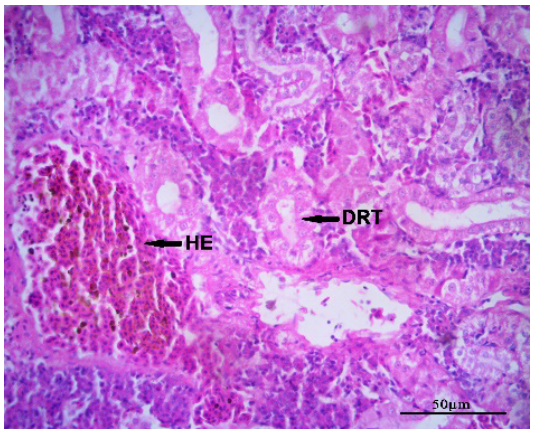

Fig. 7. Histological section of the kidney of *Pangasianodon hypophthalmus* (control).

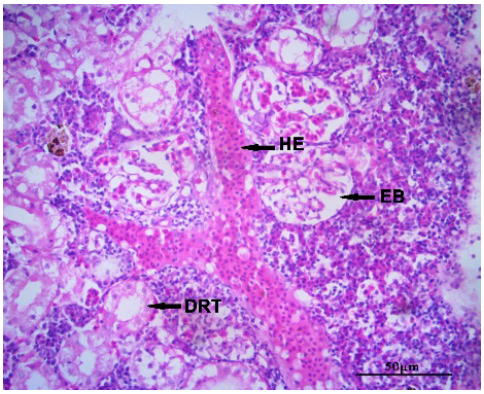

Fig. 8. Histological section of the kidney of *Pangasianodon hypophthalmus* exposed to 0.04 ppm lambda-cyhalothrin.

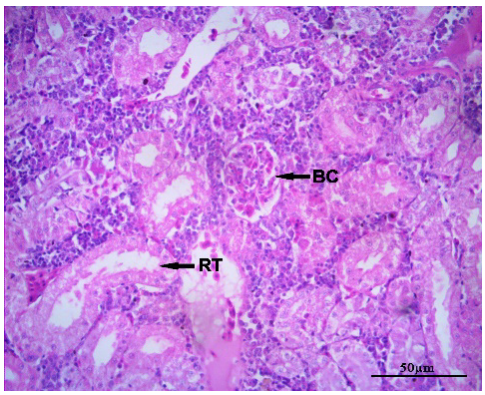

Fig. 9. Histological section of the kidney of *Pangasianodon hypophthalmus* exposed to 0.08 ppm lambda-cyhalothrin.

DISCUSSION

Pyrethroid insecticides such as lambda-cyhalothrin are widely used because of their comparatively low toxicity to birds and mammals but are highly toxic to fish. In the present investigation, acute exposure to lambda-cyhalothrin resulted in noticeable alterations in several haematological parameters, reflecting toxic stress in the exposed fish. Haematological parameters are generally considered as reliable indicators of physiological stress and therefore commonly used as biomarkers to evaluate the adverse effects of environmental contaminants in fish (Fazio, 2019). In the present study TEC, Hb and PCV showed significant reductions in treated fish. The reduction in TEC may be attributed to erythrocyte damage and cytotoxic effects caused by lambda-cyhalothrin exposure.Similar reductions have been reported in *Gambusia affinis* (Muranli and Guner, 2011) and *Cyprinus carpio* (Chatterjee *et al*., 2021) exposed to the same pesticide. Furthermore, the reduction in haemoglobin and PCV observed in the present study may indicate anaemic conditions resulting from inhibition of erythropoiesis, disruption of haemsynthesis and increased erythrocyte destruction under toxic stress (Adhikari *et al*., 2004). Similar changes in haemoglobin and PCV were reported in *Labeo rohita* exposed to lambda-cyhalothrin (Dhanya and Sushama, 2018) and in *Oreochromis niloticus* exposed to cypermethrin, another pyrethroid pesticide (Yaji *et al*., 2012). Alterations were also observed in erythrocyte indices. The present study demonstrated significant increases in MCV and MCH at the higher exposure concentration of lambda-cyhalothrin. These changes may indicate disturbances in erythropoiesis and erythrocyte physiology resulting from pesticide-induced stress. Comparable increases in MCV and MCH have also been reported in *Prochilodus lineatus* exposed to the pyrethroid insecticide cypermethrin (Parma *et al*., 2007). In contrast, MCHC showed a slight increase in the exposed groups, although the differences were not statistically significant. The exposed fish also exhibited a significant reduction in TLC compared to control, which may be due to reduced leukocyte production or increased destruction of leukocytes under pesticide-induced stress, as previously reported in fish exposed to pyrethroid pesticide (Jee *et al*., 2005; Bej *et al*., 2021).

Histopathology is considered one of the most effective tools for assessing the health status of fish populations and serves as a reliable indicator of aquatic ecosystem health in biomonitoring studies (Nikalje *et al*., 2012). Histological alterations induced by pesticide exposure provide valuable information regarding the severity of toxic effects in aquatic

organisms. Damage to vital organs can adversely affect growth, metabolism, reproduction, immunity, and survival of fish (Rohani, 2023).In addition to haematological alterations, acute exposure to lambda-cyhalothrin also produced marked histopathological changes in the vital organs of *P. hypophthalmus*.

The gills are highly susceptible to pesticide toxicity because of their direct and continuous contact with the surrounding aquatic environment and their large respiratory surface area (Au, 2004). Histopathological lesions such as epithelial lifting, necrosis, oedema, clubbing, telangiectasis, haemorrhage, lamellar fusion, and hyperplasia have been widely reported following pesticide exposure and are known to impair respiration, ion regulation, and gas exchange (Cengiz and Unlu, 2006; Velmurugan *et al.,* 2007). The gill lesions observed in the present study are consistent with these pathological responses and indicate impairment of normal gill function. Similar alterations have been reported in *Cyprinus carpio* exposed to deltamethrin, where oedema, necrosis, and hyperplasia were observed (Cengiz, 2006). Likewise, *Sarotherodon melanotheron* juveniles exposed to lambda-cyhalothrin exhibited various degrees of gill damage, including necrosis, pigment deposition, lesions, and inclusion bodies (Alalibo *et al*., 2019), supporting the present findings.

The liver plays a central role in detoxification and biotransformation of xenobiotics and is therefore particularly vulnerable to pesticide-induced injury (Soufy *et al.,* 2007). The severity of hepatic lesions depends on the type, concentration, and duration of contaminant exposure (Barhoumi and Messaoudi, 2012). Histopathological alterations in the liver are widely recognized as reliable biomarkers of environmental contamination in fish (Fernandes, Rocha and Salgado, 2008). The hepatic lesions observed in the present study suggest cellular degeneration and impaired liver function following acute lambda-cyhalothrin exposure. Similar hepatic alterations have been reported in *Clarias batrachus* exposed to lambda-cyhalothrin, where hepatocellular degeneration, vacuolation, and nuclear alterations were observed (Singh *et al.,* 2015). Likewise, lambda-cyhalothrin-induced hepatotoxicity characterized by degenerative changes and tissue damage has also been reported in *Clarias gariepinus* (Ezenwosu *et al*., 2020). Comparable hepatic alterations, including pyknotic nuclei, hepatocellular degeneration, and cytoplasmic vacuolation, have also been reported in *Mystustengara* exposed to the pyrethroid cypermethrin (Haque *et al.,* 2018) and in *Cyprinus carpio* exposed to cyfluthrin (Sepici-Dincel *et al*., 2009). These findings suggest that pyrethroid insecticides produce consistent pathological alterations in fish liver, indicating impairment of hepatic function following toxic exposure.

The kidney is essential for osmoregulation, excretion of metabolic wastes, and elimination of xenobiotics, making it highly susceptible to toxic injury (Ortiz *et al.,* 2002). Consequently, renal histopathology is considered an important indicator of environmental contamination. The renal alterations observed in the present study indicate impairment of renal architecture and function following acute lambda-cyhalothrin exposure. Similar renal lesions have been described in *Clarias batrachus* exposed to lambda-cyhalothrin, where vacuolation of renal tubules, atrophic changes, reduction in nuclei, and pyknotic nuclei were reported (Singh *et al*., 2015). Comparable histopathological alterations in the kidney, including degeneration of renal tubules, glomerular damage,

Table 1. Haematological parameters. Values are expressed as mean ± SD (n = 3). Means within a row bearing different superscript letters differ significantly (P < 0.05) according to one-way ANOVA followed by Duncan's Multiple Range Test (DMRT).

Haematological parameters	Concentrations		
	Control	0.04ppm	0.08ppm
TEC ($\times 10^{12}$ cells L^{-1})	2.40±0.05^{a}	2.14±0.06^{b}	1.54±0.18^{c}
Hb (g dL^{-1})	12.45±0.08^{a}	10.53±0.08^{b}	10.18±0.06^{c}
PCV (%)	36.34±0.99^{a}	30.93±1.55^{b}	28.23±0.92^{c}
MCV (fL)	151.11±6.02^{b}	144.23±3.39^{b}	184.62±22.64^{a}
MCH (pg)	51.80±1.00^{b}	50.24±0.55^{b}	66.64±7.43^{a}
MCHC (g dL^{-1})	33.96±0.48^{a}	35.44±3.79^{a}	36.08±1.35^{a}
TLC ($\times 10^{9}$ cells L^{-1})	10.59±0.08^{a}	8.30±0.28^{c}	8.66±0.02^{b}

and structural disorganization, have also been reported in *Cirrhinus mrigala* following acute exposure to lambda-cyhalothrin (Velmurugan *et al*., 2007). Likewise, *Oreochromis niloticus* exposed to the pyrethroid cypermethrin exhibited glomerular shrinkage, degeneration of renal tubules, and other structural alterations in kidney tissues (Majumder and Kaviraj, 2022). Collectively, these findings indicate that pyrethroid insecticides consistently induce structural damage to renal tissues, resulting in impaired kidney function in exposed fish.

CONCLUSION

The findings of the present study indicated that acute exposure to lambda-cyhalothrin induced significant haematological and histopathological alterations in *Pangasianodon hypophthalmus*. Haematological parameters, including total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and total leucocyte count (TLC), showed significant changes following exposure to the test concentrations, indicating physiological stress and impaired health status. Histopathological analysis revealed structural alterations in the gills, liver, and kidney, suggesting the susceptibility of these organs to pesticide-induced damage. These findings suggest that haematological and histopathological responses can serve as reliable biomarkers for assessing pesticide toxicity in fish. Furthermore, the results indicate that lambda-cyhalothrin contamination may adversely affect non-target aquatic organisms, highlighting the importance of its judicious use and continuous environmental monitoring.

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Conflict of interest

The authors declare noconflict of interest.

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